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DETERMINATION OF BISPHENOL A DIGLYCIDYL ETHER CONTENT IN FOODS FROM LACQUERED CANS

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ABSTRACT

Canned foods are increasingly used in food packaging. Packaging serves mainly to preserve, inform and sell foodstuffs. In order to avoid migration issues of chemical compounds from tin cans to foods, covering internal surface of the tin cans with epoxyphenolic and organosol resins is widespread. However, monomers like Bisphenol A Diglycidyl Ether (BADGE), number among the constituents of these resins capable of migrating to foods. This chemical compound (BADGE) is highly toxic not only for the immune, reproductive and hepatic systems but also for biomolecules such as DNA, nucleic acids, proteins, and hormones. Simulation tests of migration can be used to assess the significance of BADGE migration. For this study, the migration and degradation tests were realized with metallic sheets and cans lacquered with epoxyphenolic or organosol resins. BADGE concentrations were determined by High Performance Liquid Chromatography (HPLC) using a Hewlett Packard HPLC chromatograph 1050 serial equipped with an injection valve of 50 µL, a Hewlett Packard spectrophotometric UV detector serial 1050 and a Hewlett Packard integrator serial 3396. BADGE detections were made at 275 nm and compounds were separated on a LiChrospher 100 RP-18 (Merck, 250 x 4 mm I.D., 5 µm) column protected by a guard LiChrospher 100 RP-18 (Merk, 5 µm) column. The mobile phase was a mixture of methanol, water, and dichloromethane (CH_3OH-H_2O-CH_2Cl_2) according to 50%-20%-30% proportion of solvents and the flow was 1 $mL.min^{-1}$. The content in BADGE ranged from 3 to 37 $\mu g.L^{-1}$. These concentrations were only slightly influenced by the storage conditions (duration and temperature). The highest concentrations were found in distilled water from cans analyzed just after sterilization and the lowest concentrations in distilled water from cans stored at least one day before analyses. BADGE degradation tests in aqueous environment provided an explanation to the lowest concentrations of BADGE in lacquered cans. Finally, the number of sterilization also proved to be critically important for the reduction of BADGE content in cans.

Key words: Epoxyphenolic resin, BADGE, Can, Food

INTRODUCTION

The epoxyphenolic resins are used to protect, lessen or do away with the interactions between can and foods [1,2,3]. These lacquers must not only ensure some inertia but also avoid altering the organoleptic properties of foods. However, migrations of some monomers from lacquers to foods have been observed. In toxicology, the most important of these monomers are Bisphenol A (BPA) and Bisphenol A Diglycidyl Ether (BADGE). Skin attacks, allergy, asthmatic and inflammatory reactions have been shown by toxicological studies [4,5,6,7,8]. Other genotoxical effects and even reproductive system disruption have also been reported [9]. In view of the toxicity of Bisphenol A Diglycidyl Ether, the European Council fixed the limit of the specific migration of this compound in food or water at 0.02 mg/kg and the maximal content of residual BADGE allowed in packaging at 1 mg/kg [10]. Thus, it appears essential to assess BADGE content in lacquered canned foods.

EXPERIMENTAL

Chemicals and reagents

Methanol (Fisher), isooctane (Merck), dichloromethane (Carlo Erba) and distilled water used were HPLC grade. Resin of 2.2'-bis [4-(2.3-epoxypropyloxy) phenyl] from European Certification Commission office was also used.

Instrumentation

BADGE concentrations were determined by High Performance Liquid Chromatography (HPLC) [11]. A Hewlett Packard HPLC chromatograph 1050 serial was used. It was equipped with an injection valve of 50 µL, a Hewlett Packard spectrophotometric UV detector serial 1050 and a Hewlett Packard integrator serial 3396. BADGE detections were made at 275 nm wavelength and compounds were separated on LiChrospher 100 RP-18 (Merck, 250 x 4 mm I.D., 5 µm) column protected by a guard LiChrospher 100 RP-18 (Merk, 5 µm) column. The mobile phase was a mixture of methanol, water, and dichloromethane (CH_3OH-H_2O-CH_2Cl_2) according to 50%-20%-30% proportion of solvents and the flow was 1 mL.min^{-1}.

Extraction of BADGE

A Supelco LC18 cartridge containing 1g of resin was conditioned in accordance with the following sequence: methanol (CH_3OH, 20 mL), dichloromethane (CH_2Cl_2, 20 mL) and H_2O (2 mL). After that, the sample was first injected in the cartridge and then the cartridge was twice eluted with a mixture of CH_3OH-CH_2Cl_2 at different proportions (2 mL v/v then 2 mL v/3v). The extract obtained was evaporated under nitrogen gas and recovered in 1 mL of methanol and then stored at -20°C until analysis. The extract was diluted in the mobile phase before HPLC analysis.

Quality assurance of the results

The quality control of HPLC and BADGE extraction was determined according to French normalization method and included linearity, repeatability, reproducibility and extraction yield [12]. The linearity was tested from 0 to 40 µg.L^{-1} with 5 points of the

standard curve: 0.2, 1, 10, 20 and 40 µg.L^{-1}. Five analyses were performed for each point. The repeatability and reproducibility tests were realized with standards of BADGE at 20 and 40 µg.L^{-1}. Standard solutions were prepared in the mobile phase and 50 µL of these standard solutions were injected in the chromatograph. Five tests were done for the repeatability and fifteen for the reproducibility. The extraction yield was assessed by adding definite concentrations of BADGE (1, 10 and 100 mg) to distilled water. After mixing, BADGE was extracted according to the extraction procedure previously described. Triplicate assays were done for each concentration of BADGE added to distilled water. The detection and quantification limits were determined with a blank descended from extraction and equal:

Limit of Detection = $m_b + 3\sigma$
Limit of Quantification = $m_b + 10\sigma$
(m_b = average concentration with the blank; σ = Standard deviation of blank values, n=30)

The results of the validation tests are presented in Table 1. The variation coefficients for repeatability and reproducibility were similar and ranged between 2.5 and 4.2% for repeatability and 2.6 and 4.1% for reproducibility. The extraction yields were between 82.8 and 90.3%. The limit of detection was 0.2 µg.L^{-1} and the limit of quantification was 0.5 µg.L-1.These results account for the reliability of the technique that was used for BADGE extraction and analysis.

Migration tests of BADGE
Migration tests were realized with metallic sheets and lacquered cans. The cans were provided by a factory located in the region of Meurthe and Moselle in France and specialized in the manufacture of tin cans for foodstuffs packaging.

Characteristics of the metallic sheets
The circular metallic sheets used had a diameter of 1.7 dm and an area of 2.3 dm^2. The specifications of lacquers used were:

- Epoxyphenolic lacquer: 5.5 g/m^2;
- Organosol lacquer: 18g/m^2.

Characteristics of the lacquered cans
The tin cans used were “3 pieces” (a cylindrical body, a bottom and a cover). Each can was of the following dimensions: 1.10 dm height, 0.75 dm radius and 3.5 dm^2 area. The specifications of the resins were:

- body and covert: lacquer monocoat 5.5 g/m^2;
- bottom: lacquer monocoat 7 g/m^2;
- rechampi: acrylic lacquer 10 g/m^2.

Three types of cans were used:

- non set and non sterilised cans (CNSeSt),
- set and non sterilised cans (CSeNSt),
- set and sterilised cans (CSeSt).

The migration tests were done according to the European directive 85/572/CEE [13]. The influence of storage conditions (temperature and duration) and sterilization (temperature and number of sterilization) on BADGE migration was determined. The influence of storage conditions (temperature and duration) on BADGE degradation was also determined.

Influence of storage temperature on BADGE migration

The three types of cans filled with 375 mL of distilled water were stored for 10 days under the temperatures of 4, 20, 40 and 60°C. After storage, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of storage duration on BADGE migration

The three types of cans filled with 375 mL of distilled water were stored for 1, 5 and 10 days under 20 and 40°C. After storage, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of sterilization temperature on BADGE migration

Metallic cans lacquered with epoxyphenolic resins and filled with 375 mL of distilled water were set and sterilized at six different temperatures (100, 105, 110, 115, 121 and 130°C) for 20 minutes. After cooling, the content of each metallic can was extracted in accordance with the extraction method previously described. Analyses were done in triplicate.

Influence of the number of sterilization on BADGE migration

A metallic sheet lacquered with organosol resin was immersed in an Erlenmeyer filled with 500 mL of distilled water. The Erlenmeyer covered with an aluminium film was four times successively sterilized in an autoclave LX Lequeux for 20 minutes at 121°C. After each sterilization, distilled water was collected and the metallic sheet was rinsed, then immersed again in an Erlenmeyer containing 500 mL of distilled water for another sterilization in the autoclave. Five different measurements of BADGE concentration in distilled water were done after each sterilization.

Influence of storage duration and temperature on BADGE degradation

Solutions of 100 $mg.L^{-1}$ of BAGDE were prepared by adding 50 mg of BADGE to 500 mL of distilled water in round glass bottles. The bottles were tightly closed and stored for 10 days at 4, 20, and 40°C. BADGE concentrations were determined from the first to the tenth day in triplicate for each day of storage.

RESULTS

Influence of storage temperature on BADGE migration

Concentrations of BADGE in distilled water were very low (about 3µg.L^{-1}) for all the different types of cans (2 µg.L^{-1} for CSeNSt, 2 µg.L^{-1} for CSeSt and 2.6 µg.L^{-1} for CNSeSt) (Figure 1).

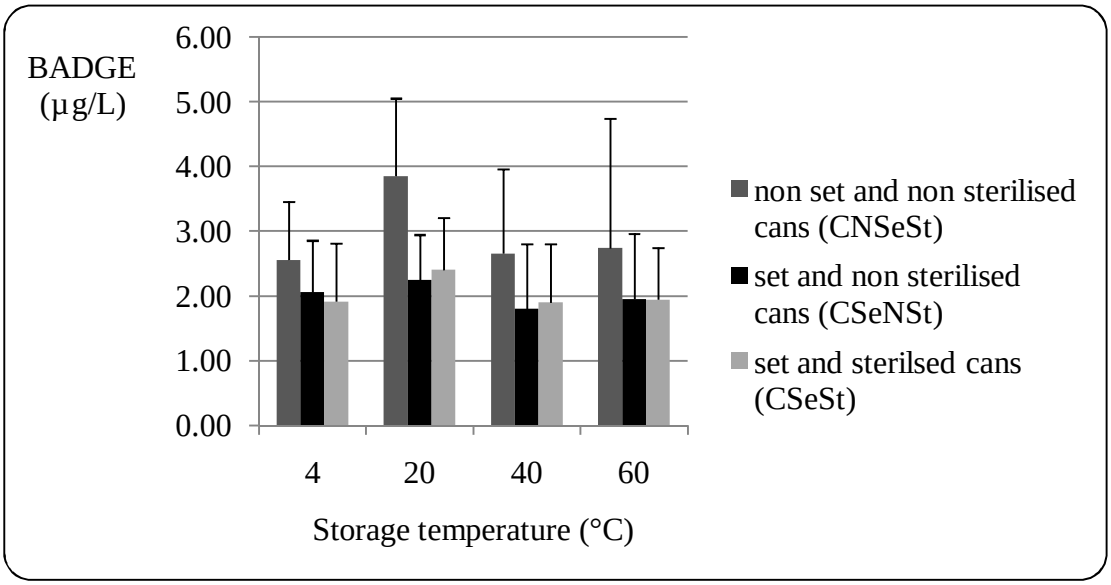

Figure 1: Influence of storage temperature on BADGE migration

Influence of storage duration on BADGE migration

The results of the influence of storage duration on BADGE migration are shown in Figure 2 (2a, 2b and 2c). For all types of cans, BADGE concentrations were about 3µg.L^{-1} and were only slightly influenced by storage duration.

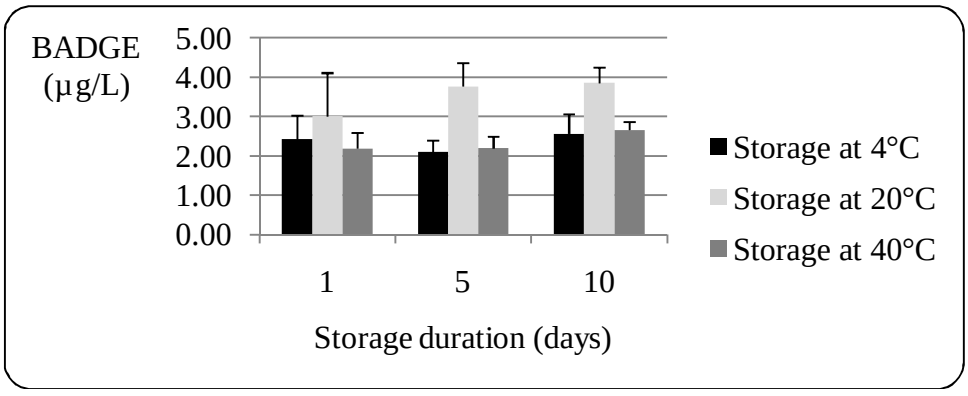

Figure 2a: Effect of storage duration on non-set and non-sterilised cans (CNSeSt)

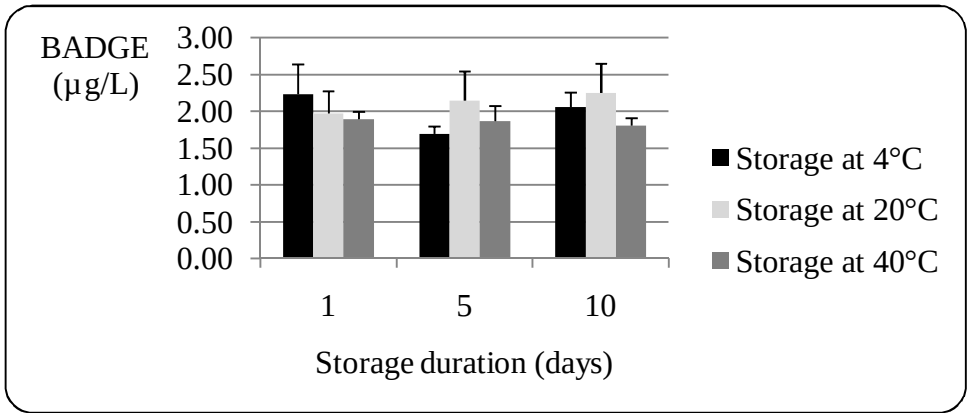

Figure 2b: Effect of storage duration on set and non-sterilised cans (CSeNSt)

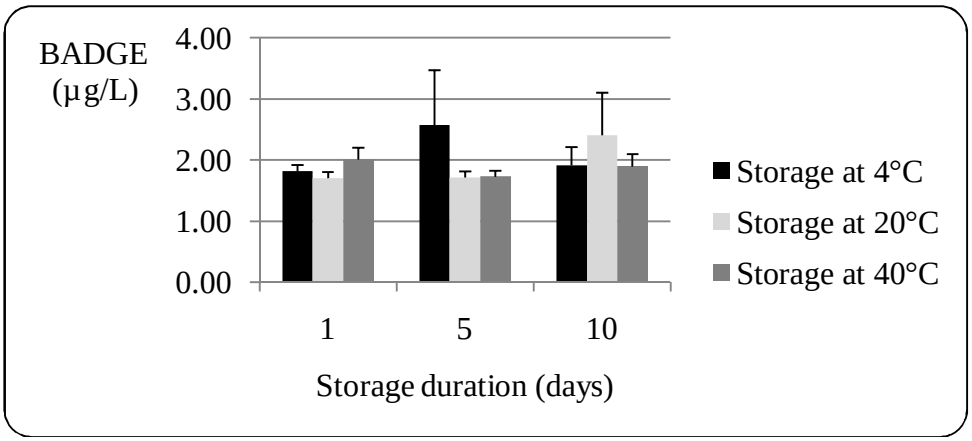

Figure 2c: Effect of storage duration on set and sterilised cans (CSeSt)

Influence of sterilization temperature on BADGE migration

The results of the influence of sterilization temperature on badge migration are presented in Figure 3. BADGE concentrations in cans ranged between 13 and 37 $\mu g.L^{-1}$. The highest concentrations were determined at 100°C while the lowest were at 130°C.

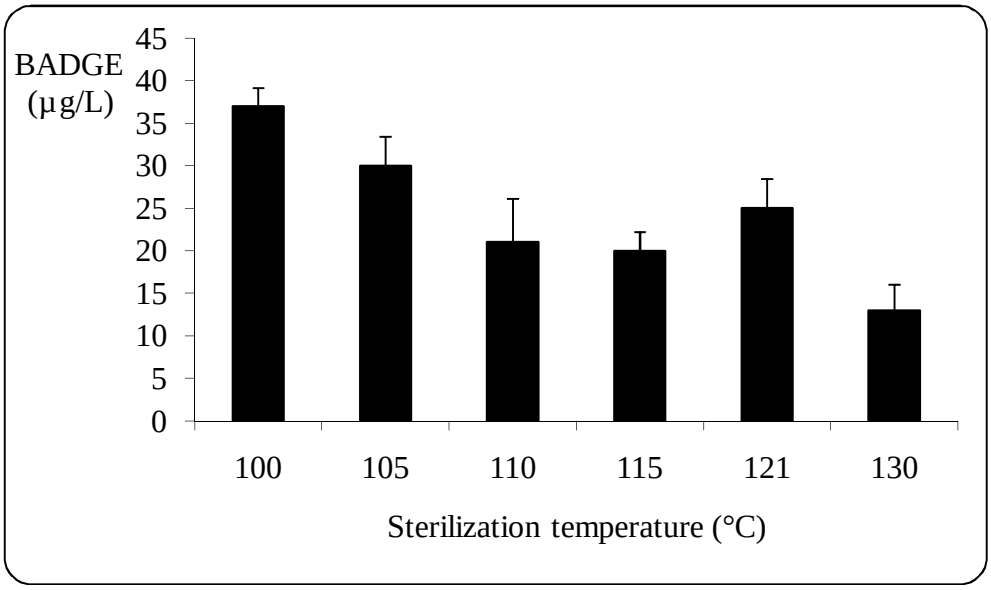

Figure 3: Influence of cans sterilisation temperature on BADGE migration

Influence of the number of sterilization on BADGE migration

The influence of the number of sterilization on BADGE migration is presented in Table 2. BADGE was only found in distilled water from the two first sterilizations.

Influence of storage temperature and duration on BADGE degradation

The influence of storage temperature and duration on BADGE degradation is presented in Figure 4. Data obtained showed a decrease in BADGE concentration with the storage temperature and duration time.

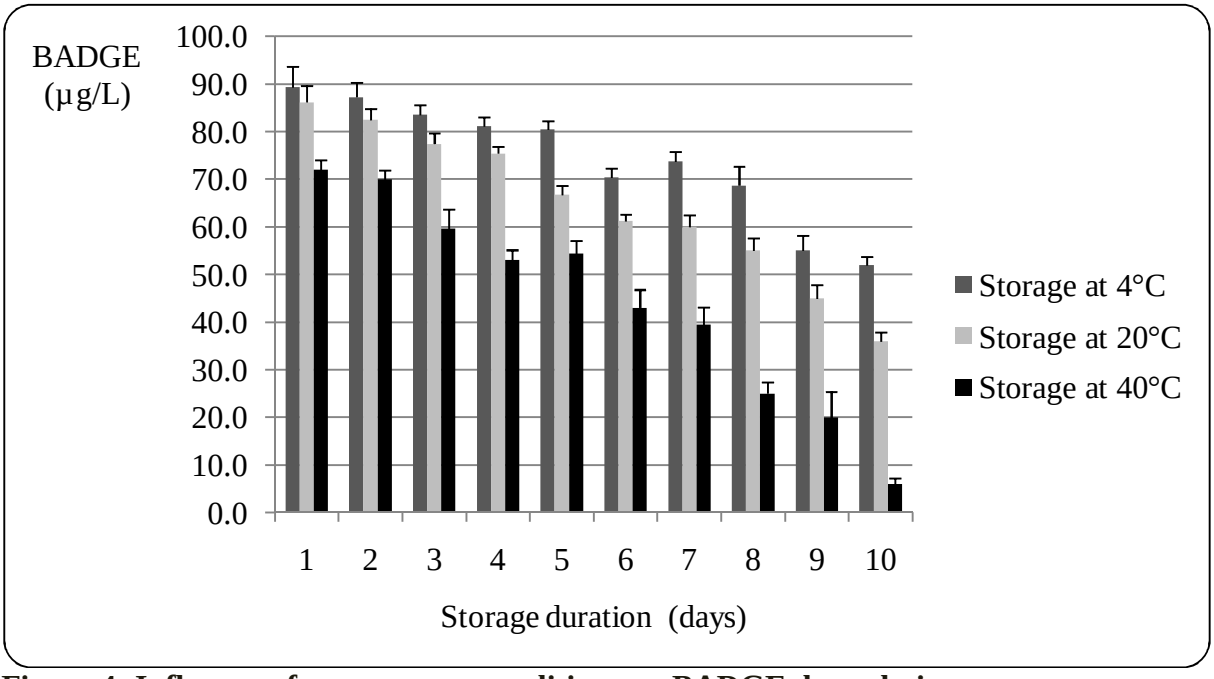

Figure 4: Influence of cans storage conditions on BADGE degradation

DISCUSSION

BADGE concentrations were only slightly influenced by an increase in storage temperature and duration. The European Community [10] set the limit value of BADGE specific migration at 20 µg.kg^{-1} of food or water. This limit value is seven times higher than the concentrations in our study. Except for some samples of sardines and anchovies (concentrations superior to 1 mg.kg^{-1}), data from our study were similar to those obtained in England by the Ministry of Agriculture, Fishing and Food during a survey about BADGE content in canned foods [14].

As for the influence of the temperature of sterilization, the lowest concentration was obtained at 130°C (13 µg.L^{-1}) and was twice lower than the concentrations determined under the other sterilization temperatures. For the other sterilization temperatures, BADGE concentrations were similar and ranged from 22 to 37 µg.L^{-1}. Concentrations of BADGE in this experiment were nine times approximately higher than those determined in the study on the influence of storage conditions (temperature and duration) on BADGE migration. This trend was due to the fact that, in this experiment, samples were analyzed one hour after cans sterilization whereas in the previous experiments the cans were stored at least one day before analyses. In fact, as observed by some authors, the BADGE is hydrolysed during the conditioning of cans [15,16,17].

With regard to the number of sterilization, the quantity of BADGE released in distilled water by the metallic sheet ranged from 10 µg.L^{-1} after the first sterilization to concentrations under the detection limit after the fourth sterilization. The major part of BADGE was eliminated only after two sterilizations at 121°C during 20 minutes. To sum up, the number of sterilization is critically important when it comes to do away with resins monomers capable of migrating to foods.

As regards BADGE degradation tests, the results showed that during the ten days of storage, BADGE concentrations moved from 100 to 52 mg.L^{-1} for storage at 4°C, from 100 to 36 mg.L^{-1} for storage at 20°C and from 72 to 6 mg.L^{-1} for storage at 40°C. The corresponding percentages of degradation after ten days of storage at 4, 20 and 40°C were respectively 48, 74, and 94%. The study of the influence of storage temperature and duration on BADGE degradation seems to corroborate data from other studies which established a decrease in BADGE concentration with the duration time of storage (more marked after 10 days) [17,18,19]. Indeed, Philo and collaborators [19] reported that 90 to 100% of BADGE content was released during migration tests in aqueous environment. They mentioned that the percentages of BADGE degradation in distilled water or acetic acid (3%) stored ten days were 100% at 40°C and between 95 and 100% at 100°C.

CONCLUSION

BADGE degradation in aqueous environment provided an explanation to the infinitesimal concentrations of BADGE in lacquered metallic cans. Besides, the more the cans were sterilized, the more BADGE was released. Thus, it clearly appears that food companies should sterilize several times tin cans before conditioning food.

Table 1:Results of quality assurance

Linearity	
Standard range: 0.2 µg.L^{-1}, 1 µg.L^{-1}, 10 µg.L^{-1}, 20 µg.L^{-1}, 40 µg.L^{-1}	
Standard equation: y = 177883X - 70135	
Coefficient of determination: R = 0.9897	
Repeatability (n=5)	***Variation coefficient* (%)**
20 µg.L^{-1}	4.2
40 µg.L^{-1}	2.5
Reproductibility (n=15)	***Variation coefficient* (%)**
20 µg.L^{-1}	4.1
40 µg.L^{-1}	2.6
Extraction yields (n=3)	***Variation coefficient* (%)**
1 mg	82.8 ± 2.8
10 mg	88.1 ± 3.7
100 mg	90.3 ± 3.0
Limit of detection (n=30)	0.2 µg.L^{-1}
Limit of quantification (n=30)	0.5 µg.L^{-1}

Table 2: Influence of the number of sterilization on BADGE migration (Concentration: µg.L^{-1}; n = 5)

Sterilisation number	S1	S2	S3	S4
Bisphenol A concentration	10 ± 4	1 ± 1	< LD	< LD

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